## REMARKS

Claims 1, 2, 4-19 and 21-55 are pending. Claims 15, 28, and 30-37 are withdrawn from consideration, but must be considered once an allowable product claim has been indicated. To that end, they have been amended in concert with the amendments to the product claims. Claims 1, 2, 4-14, 16-19, 21-27, 29 and 38-55 have been rejected. Claims 1, 6, 8, 16, 19, 22, 30, 38, 40, 41, 43, 44, 46, 47, 49, and 53-55 have been amended. Support for the amendments and new can be found in the disclosure at page 7. Claims 39, 42, 45, 48, and 50-52 have been canceled. Claims 1, 2, 4-19, 21-38, 40, 41, 43, 44, 46, 47, 49 and 53-55 remain in the case.

Applicants would like to thank Examiner Helms for the courtesy of an interview on May 13, 2002. At the interview, language was discussed to overcome the rejection under Section 103. The present claim amendments reflect that discussion.

Claims 19-26 and 53-55 are rejected under Section 103(a) based on Shih *et al.* U.S. 5,057,313) in view of Leung *et al.*, *Int. J. Cancer*, 60:534-538 (1995) ("Leung I") and Qu *et al.*, *Glycobiology*, 7:803-809 (1997). The examiner urges that Shih discloses oxidizing a carbohydrate of an antibody to produce ketones and conjugating drugs and toxins to the oxidized antibody. The examiner admits that Shih does not teach glycosylation at the HCN1, HCN5 or Vκ-N site, but alleges that these deficiencies are made up for by the teaching of Leung and Qu.

As discussed during the May 14th interview, the claims are being amended to recite that "the reactive ketone group is not introduced by oxidation." It is clear that the present application discloses an alternative to introducing reactive groups by oxidizing a sugar. For example, page 2 of the specification discusses Leung *et al.*, *J. Immunol.* 154: 5919 (1995) ("Leung II"), which is exemplary of methods that oxidize a carbohydrate, as follows:

> in order to conjugate at these carbohydrates, the ribose rings must be chemically oxidized to generate reactive aldehyde groups. Aldehyde groups thus formed can be covalently bonded to the amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups

> attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide.

The present invention provides glycosylated antibodies that do not require this oxidation, as an alternative to methods like that disclosed in the Leung II and Shih. The present method clearly goes directly from introduction of a ketone derivative onto an antibody to reacting the "resulting antibody" with a ketone reactive group, *i.e.*, the reactive ketone group is introduced as a conjugate to a non-oxidized sugar.

The portion of Shih that is cited by the examiner actually is a reference to a published application by McKearn (EP 88,695), which discloses "a method for preparing antibody conjugates which involves oxidizing the carbohydrate portion of the antibody and linking compounds with free amine groups to the resultant carbonyls (aldehyde and/or ketone groups) by Schiff base formation. The harsh oxidation used to open the ring and thereby generate the carbonyl groups, especially where complete oxidation of all carbohydrate residues is desired, and the harsh reducing environment used to stabilize the Schiff base conjugate, both may impair the biological activity of the molecule. By contrast, the antibodies according to the present invention already have a reactive ketone group as a side chain on the carbohydrate used in the glycosylation of the antibody, which is produced by the transfected host cell's biosynthetic machinery. Shih (McKearn) does not disclose a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site that is not introduced by harsh oxidation. In particular, Shih (McKearn) does not disclose such a glycosylated antibody prepared by the method of claim 1.

The addition of Leung I and/or Qu to Shih (McKearn) would not have suggested the invention as presently claimed. Leung discloses a glycosylation site in the Vκ domain and that this site can be used for conjugations. Leung I references other articles which disclose the conjugation technique. Like Shih (McKearn), these entail chemical oxidation of the rings to generate reactive aldehyde groups, which then can be covalently bonded to the

amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide, hence Leung's disclosure that an average of 2 to 6 chelators such as DTPA could be conjugated.

Qu teaches the compositions and sequences of CH1-appended carbohydrates from two antibodies, hLL2HCN1 and hLL2HCN5, as determined by fluorophore-assisted carbohydrate electrophoresis (FACE). The structural profile of hLL2HCN1-carbohydrates revealed that about 2-4 hexose rings in an oligosaccharide chain are available for periodate oxidation. Therefore, a maximum of 8-16 aldehyde groups on average can be generated from the carbohydrate side chains of each hLL2HCN1 F(ab')2 fragment. With the average size of hLL2HCN5-carbohydrate being 3-4 monosaccharide residues larger than that of HCN1, a higher number of maximum achievable aldehyde groups for hLL2HCN5 is expected.

Qu does not overcome Shih's failure to teach conjugation methods that use introduced reactive ketone groups on the side chains of the glycosylation carbohydrates, as opposed to chemical oxidation of the carbohydrate ring and subsequent covalent bonding of the thus-generated aldehyde groups to the amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide. As discussed above, chemical oxidation to generate carbonyl groups has significant adverse consequences. When harsh conditions are used to generate the maximum number of such groups, the three-dimensional structure of the antibodies is altered and the immunoreactivities of the antibodies may suffer. And under milder chemical conditions, only 1.6 and 3 molecules of DTPA are conjugated to the F(ab')2 of hLL2HCN1 and hLL2HCN5 sites, respectively, probably due to inefficient oxidation of hexose rings under these conditions.

All of the references cited by the examiner disclose the use of harsh oxidation conditions to derivatize a glycosylated antibody. The antibodies according to the present

invention, on the other hand, have a reactive ketone group on a side chain, and are produced by the transfected host cell's biosynthetic machinery. None of the cited references disclose or suggest a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, and more particularly a glycosylated antibody prepared by the method of claim 1. In accordance with the present invention, these antibodies are made recombinantly by a transfected host cell. The host cell's biosynthetic machinery converts the antibodies so that they have a reactive ketone group. Reconsideration and withdrawal of the rejections under Section 103 based on Shih, in view of Leung and Qu is respectfully requested.

Claims 1, 2, 4-14, 16-19, 21-27, 29, 38, 41, 44, 47, 50, and 53-55 are rejected under the first paragraph of Section 112. In response to applicants' argument that potential glycosylation sites can be identified by computer modeling and then screened as described in Example 2, the examiner now replies that "it is not clear from the specification what of the many saccharide precursors or 'ketone derivatives' would work in the claimed method." As noted in connection with applicants' response in their last amendment to a Section 112, second paragraph, rejection, the present invention relates to glycosylated antibodies. This context provides the skilled artisan with guidance on what ketone derivatives "work in the claimed method," as there are a very limited number of saccharides that are used in glycosylation. Indeed, the excerpt from Stryer, Biochemistry that was appended to the response of December 21, 2001, showed the formulae of saccharides commonly found in oligosaccharide units of glycoproteins; these include β-L-fucose, β-D-galactose, β-D-N-acetylgalactosamine, β-D-N-acetylglucosamine, β-D-mannose, and sialic acid. The examiner's reference to "many" potential ketone derivatives is ill-founded in the context of glycosylated antibodies. In addition, applicants have amended the claims to emphasize that the saccharide precursors are "biosynthetic" saccharide precursors, as supported by page 7 of the disclosure. Accordingly, the scope of the claims as amended is fully enabled, and reconsideration and withdrawal of this rejection is requested.

Claims 39, 40, 42, 43, 45, 46, 48, 49, 51 and 52 are rejected under the first paragraph of Section 112. Applicants do not agree with the basis for this rejection, but in order to advance prosecution, claims 39, 42, 45, 48 and 51-52 have been canceled subject to applicants' right to pursue protection for this subject matter in a continuing application, and the dependencies of claims 40, 43, 46 and 49 have been amended accordingly.

Based on the foregoing amendments and remarks, all claims are believed to be in condition for allowance. Should there be any matter requiring further attention, the examiner is invited to contact the undersigned at the local telephone exchange listed below.

Respectfully submitted,

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Date

B. G McDowell Reg #31,640
for Bernhard D. Saxe
Reg. No. 28,665

**FOLEY & LARDNER**
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5109
Telephone: (202) 672-5427
Facsimile: (202)672-5399

## MARKED-UP VERSIONS OF CLAIM AMENDMENTS

Please cancel 39, 42, 45, 48, 49, and 50-52. and amend the remaining claims as follows:

1. (Twice Amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor, and]

expressing [said transfected] SP2/0 cells that are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site.

6. (Twice Amended) A method of making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,]

expressing [said transfected] SP2/0 cells that are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody into] to produce a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

8. (Twice Amended) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,]

reacting [the resulting antibody] a glycosylated antibody produced according to claim 1 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, [and] thereby conjugating said glycosylated antibody to [an] said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

16. (Twice Amended) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or Vκ domain in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP 2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,

fragmenting the resulting antibody into an antigen-binding antibody fragment, and]

reacting [the] a glycosylated antibody fragment produced according to claim 6 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said

glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

19. (Twice Amended) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, wherein said glycosylated site is in the Vκ or CH1 domain, and wherein the reactive ketone group is not introduced by oxidation.

22. (Twice Amended) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through the glycosylated site, wherein said glycosylated site is in the Vκ or CH1 domain, and wherein the agent is conjugated to a reactive ketone group on the glycosylated site that is not introduced by oxidation.

30. (Twice Amended) A method of targeting an active agent to an *in vivo* target site comprising administering an immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment [conjugated to an active agent through the or antigen-binding antibody fragment] conjugated to an active agent through a reactive ketone group on a glycosylated HCN1, HCN5 or Vκ-N glycosylation site and not as a conjugate to an oxidized sugar.

38. (Amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor, and]

expressing [said transfected] SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, so that

they produce an N-glycosylated antibody having a reactive ketone group on the glycosylated site.

40. (Amended) A method according to claim [39] 38, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

41. (Amended) A method making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,]

expressing [said] SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody into a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

43. (Amended) A method according to claim [42] 41, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

44. (Amended) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transfected SP2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,]

reacting [the resulting antibody] a glycosylated antibody according to claim 38 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, [and] thereby conjugating said glycosylated antibody to [an] said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

46. (Amended) A method according to claim [45] 44, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

47. (Amended) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

[transfecting SP2/0 cells with a vector encoding an antibody having one or more N-glycosylation sites selected from the group consisting of HCN1, HCN5 the Vκ-N in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

expressing said transferred SP 2/0 cells so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site,

fragmenting the resulting antibody into an antigen-binding antibody fragment, and]

reacting [the] a glycosylated antibody fragment according to claim 41 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

49. (Amended) A method according to claim [48] 47, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

53. (Amended) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and Vκ-N, and wherein the reactive ketone group is not introduced by oxidation.

54. (Amended) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through a reactive ketone on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and Vκ-N, and wherein the reactive ketone group is not introduced by oxidation.

55. (Amended) A glycosylated antibody having a reactive ketone group on a glycosylated site, prepared by a method as recited in claim 1, wherein the reactive ketone group is not introduced by oxidation.